the same time conditions are favorable for the esterification of cholesterol caused by the presence of bile salts, fatty acid, and cholesterol esterase also entering from the lumen. There results an increased synthesis of cholesterol esters, which then pass into the central lacteal. The cholesterol esters do not pass into the lymph alone but rather are transferred along with triglyceride, phospholipide, free cholesterol, and protein. All of these substances occur together as the chylomicron when cholesterol is absorbed. The exact site of this chylomicron formation is unknown, but free cholesterol is probably drawn from the free cholesterol pool in the mucosa for this formation. Consistent with this proposed mechanism is the observation that during cholesterol absorption there are increases in the phospholipide and triglyceride contents of lymph. Also when fat alone is fed, there is an increase in the cholesterol of lymph. If the mechanism presented is correct, the appearance of free cholesterol in lymph is a necessary part of the transport mechanism for the esterified cholesterol.

Finally, the appearance of "extra" cholesterol in lymph after the administration of cholesterol results from increases in the rate of processes going on at all times in the mucosa. In a way the appearance of this "extra" cholesterol may be looked upon as the result of a homeostatic mechanism for maintaining the constancy of the cholesterol fractions of the intestinal mucosa.

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Effect of Various Dietary Components on Cholesterol Metabolism

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MANY of the experiments purporting to report on cholesterol metabolism are, in fact, concerned only with changes in the serum cholesterol levels. Serum cholesterol is an important biological parameter, but if we are to consider it as the sole measure of the degree of cholesterol metabolism, it might be well to consider the variability of serum cholesterol levels in standard conditions in man and animals.

The wide fluctuations in serum cholesterol levels shown by many individuals make a base-line study mandatory when only changes in serum cholesterol values are measured as the criterion of deeper metabolic changes. Wilkinson (1) has studied the variations in human cholesterol levels over a two-year period. The range of fluctuation is wide and displays no apparent periodicity. Schube (2) observed 10 men over a 16-week period and found only three cases in which the maximum variation was less than 20%. His data are summarized in Table I. More recently Rivin and co-workers (3) studied 10 persons over periods ranging from six months to a year. Here again, the maximum deviation from the main serum cholesterol level ranged from 9 to 29% with most of the deviations between 14 and 18%. In a larger study (30 men), carried out over a shorter period (13 weeks), Gordon and Brock (4) observed similar fluctuations. In animal studies variations in serum cholesterol levels have been observed in monkeys (5, 6) and dogs (7); and a strain of rats, in which the serum cholesterol levels go through a minimum with age, has been described (8). The foregoing has been presented to show that the disposition of cholesterol throughout the animal body should be known for proper assessment of the metabolism of this sterol. In many cases serum cholesterol must serve as the sole indicator of cholesterol metabolism, but, where possible, as many other data as possible should be assembled.

TABLE I
Serum Cholesterol Variability
(Ten males, 16-week study)

Case	Mean cholesterol level	Range
	mg. %	
1	143	100–187
2	167	151–186
3	122	106–148
4	160	142–198
5	132	120–151
6	152	115–185
7	148	135–170
8	154	134–190
9	141	124–170
10	155	116–196

In the ensuing discussion many experiments will

be related to experimental atherosclerosis since a large proportion of cholesterol-feeding experiments derive from the early observations that feeding cholesterol to rabbits results in fatty deposits in their aortas (9). Presence or absence of cholesterol-containing plaques is an indication of a mode of cholesterol metabolism.

Of the various dietary components, the relationship between fat and cholesterol has been studied most extensively. Alfin-Slater and her co-workers (10) have shown that essential fatty acid deficiency in the rat causes a drop in serum cholesterol with a concomitant rise in the liver and adrenal cholesterol levels. In experiments in which only serum cholesterol levels were measured, it has been shown that dietary unsaturated fat has a hypercholesterolemic effect in rats (11, 12) and calves (13).

Our own experiments in this field were prompted by an observation that there was less fat in the livers of corn oil-fed rabbits than in animals on normal diets (14). We then tested the effect of various fats added to the diet of cholesterol-fed rabbits, with results as shown in Table II (15). This experiment pointed up several interesting facts: first, that a fat-free cholesterol diet is the most atherogenic; second, that severity of atherosclerosis could not be correlated with serum cholesterol levels. In a second experiment, using pure methyl esters of stearic, oleic, and 9,11-octadecadienoic acids, we found an inverse relationship between serum cholesterol levels and atherosclerosis as well as between atherosclerosis and iodine value of the fat (16). These data are summarized in Table III.

The most important facet of the effects of dietary fat on serum cholesterol levels is the lowering seen in humans following ingestion of unsaturated fat. Following the initial observations of Ahrens (17) and Kinsell (18), there has been a flood of publications confirming this effect of unsaturated fat. The question of whether the fat must be merely unsaturated or whether it must be rich in essential fatty acids as well is still a matter of controversy.

The serum cholesterol seems to be preferentially esterified with unsaturated acids (19, 20). Enzymic esterification of cholesterol is more rapid with unsaturated than with saturated acids (21), a finding which may partially explain the high unsaturation of serum cholesterol ester fatty acid. The feeding of unsaturated fat may enhance the circulation of cholesterol, thus making more sterol available for degradation of bile acids, the major catabolic products of

TABLE II
Effect of Dietary Fat on Cholesterol-Induced Atherosclerosis in Rabbits (I)

	Dietary Fat		
	None	Shortening[a]	Corn oil
No. rabbits	15	15	15
% Fat in diet	...	6	6
% Cholesterol in diet	3	3	3
Iodine value of fat[b]	....	72	130
Atheromata (visual grade)	3.80	3.71	2.71
Serum cholesterol (mg. %)	2000	3990	3150
Serum lipoproteins, Sf 0–20 (mg. %)	825	1263	1112
Serum lipoproteins, Sf 20–400 (mg. %)	1691	3524	3214
Liver wt. (g.)	98	101	109
Liver cholesterol (g.)	7.3	10.1	14.2

[a] Shortening used was a commercial hydrogenated vegetable oil, purchased in a local grocery.
[b] All iodine values were carried out on the material under study, using the Wijs method as described by Hawk, Summerson, and Oser in "Practical Physiological Chemistry," p. 97, 1947 edition (The Blakiston Co., Philadelphia, Pa.).

TABLE III
Effect of Dietary Fat on Cholesterol-Induced Atherosclerosis in Rabbits (II)

	Dietary Fat		
	Methyl stearate	Methyl oleate	Methyl 9,11-octadecadienoate
No. rabbits	9	9	9
% Fat in diet	6	6	6
% Cholesterol in diet	3	3	3
Iodine value of fat[a]	0	90	147
Atheromata (visual grade)	2.78	1.89	1.69
Serum cholesterol (mg. %)	2167	2337	2578
Serum lipoproteins, Sf 0–20 (mg. %)	1303	1177	922
Serum lipoproteins, Sf 20–400 (mg. %)	2577	2965	2711
Liver wt. (g.)	99	105	81
Liver cholesterol (g.)	2.6	5.7	2.2

[a] See footnote, table II.

cholesterol metabolism (22), or for excretion. In experiments designed to elucidate the disposition of cholesterol under the influence of unsaturated fat it has been shown, using cholesterol-C^{14}, that unsaturated fat enhances excretion of circulating cholesterol (23). In a similar study, using unlabelled cholesterol, it was shown that there was increased fecal excretion of both sterols and bile acids in subjects ingesting unsaturated fat (24).

Early experiments involving the effect of dietary protein on cholesterol-induced atherosclerosis in rabbits suggested differences between materials of plant and animal origin. Diets in which the protein was defatted beef (25) or casein (26) were decidedly more atherogenic than were diets containing protein of vegetable origin (26, 27). The data of Meeker and Kesten (26) are given in Table IV and show no correlation between severity of atherosclerosis and serum cholesterol levels in animals receiving different levels and kinds of protein.

TABLE IV
Effect of Type of Protein on Experimental Atherosclerosis in Rabbits

	Diets		
	Basal	Casein	Soy protein
Protein-fat-CHO (%)	15–55–5	38–39–4	39–34–3
Cholesterol (mg./day)	250	250	250
Atheromata (occurrence)	15/21	10/13	6/16
Atheromata (grade)	1.24	2.08	0.44
Serum cholesterol (mg. %)	460	305	575

In chickens, diets high in cholesterol and low in protein lead to hypercholesterolemia (28, 29, 30, 31). When the protein is soybean protein, the cholesterol levels are lower than when casein is fed (32). There are no supplementary data available in these experiments which could explain the observations, but other information may help to elucidate these findings. In rats it has been shown that the absence of labile methyl groups in the diet (choline or methionine deficiency) leads to hypocholesterolemia with concomitant accumulation of cholesterol in the liver (33, 34). Addition of 0.5% choline to a diet containing 2% cholesterol and 10% casein will cause a five-fold increase in serum cholesterol and a 50% drop in liver cholesterol (33).

In man, low-protein diets cause large drops in the serum cholesterol levels (34). Of course, it was not possible to study liver cholesterol in these cases, and no data on the excretion of sterols or bile acids are given.

Another aspect of the effect of dietary protein is the effect of sulfur-containing amino acids. In mon-

keys (35) and in rats (36) a diet rich in cholesterol and poor in sulfur-containing protein leads to hypercholesterolemia, but addition of methionine to the diet may partially prevent this effect. The hypercholesterolemic effect of diets poor in sulfur-containing amino acids may result from a block in cholesterol catabolism and excretion since it has been demonstrated that this type of sulfur deficiency inhibits formation of taurine (37) and, one may suppose, of taurine conjugated bile acids. Choline, in addition to its lipotropic effect, appears to release liver cholesterol into the blood. A similar effect of methionine without the lipotropic property (Table V), may work further to cause increased excretion of bile acids (34).

TABLE V
Effect of Methionine and Choline on Liver Lipides and Serum Cholesterol of Rats

Diet			Liver lipide	Serum cholesterol
Lard	Methionine	Choline		
(%)	(%)	(%)	(%)	(mg. %)
40	0.16	....	35.6	50
40	0.16	0.3	6.2	93
40	0.54	....	22.9	88
40	0.54	0.3	5.8	90

The effect of carbohydrate on cholesterol metabolism has been neglected until recently. It has been the custom of workers in this field to alter the proportion of fat or protein in the diet at the expense of carbohydrate with no thought of the possible consequence of the altered carbohydrate intake. Portman, Lawry, and Bruno (38) have found that, under the added influence of cholic acid, sucrose is more hypercholesterolemic than is starch. Addition of a sulfa drug to the diet causes the difference to vanish. Cholesterol-sucrose-fed chickens have almost double the serum cholesterol levels of chickens fed on cholesterol and glucose (32). Addition of an antibiotic to the diet causes no change in the serum cholesterol levels of the sucrose group but raises the levels of the glucose-fed birds (39). Table VI presents some of these results.

To elucidate further the mechanism of this effect of dietary carbohydrate, six groups of chickens were maintained on diets in which the only sourc eof carbohydrate was either glucose or sucrose (60%). Two groups were fed cholesterol (3%), two groups received added cholesterol and antibiotic (0.02%), and two received no added sterol or antibiotic. After 28 days each bird was given one oral dose of cholesterol-4-C^{14} (75,000 counts/min.), and after two days the birds were killed. The blood, liver, carcass, gut, and feces of each animal were assayed for radioactivity. In the case of all the samples except the blood, the material was dissolved in a strong base and extracted so as to yield an acidic and an unsaponifiable fraction. These fractions were not further characterized. Between 85 and 93% of the administered isotope was recovered in each group. In general, the sterol-sucrose groups retained more of the label. In the antibiotic-fed birds there was less radioactivity in all the acidic fractions, suggesting inhibition of intestinal conversion to bile acids and other acidic material.

TABLE VI
Cholesterol-Carbohydrate Feeding to Chickens

Group	Glucose	Sucrose	Cholesterol	Aureomycin	Serum cholesterol
	(%)	(%)	(%)	(%)	(mg. %)
1	60	....	....	.....	242 ± 23
2	....	60	....	.....	179 ± 19
3	60	....	3	.....	486 ± 118
4	....	60	3	.....	752 ± 133
5	60	....	3	0.02	624 ± 111
6	....	60	3	0.02	744 ± 142

In considering the data presented above, the role of intestinal bacteria in the disposition of dietary cholesterol assumes considerable importance. It is generally agreed that the major catabolic pathway of cholesterol is by way of the bile acids, and inasmuch as bacteria play a role in the excretion of bile acids, they must influence cholesterol metabolism. In germ-free animals this mechanism for cholesterol disposition is disrupted, consequently the half-life of bile acids in such animals is greatly increased (40). It has also been shown that administration of antibiotics or sulfa drugs to rats results in lessened excretion of bile acids (40) as well as a change in the form in which they are excreted (41).

The effects of dietary components on cholesterol metabolism suggest that their influence is the result of changes they may exert on the the intestinal flora. In the case of carbohydrate (39), antibiotics affect the comparative hypocholesterolemic property of dietary glucose in chickens, but the cholesterol levels in the sucrose-fed birds are unaltered. This suggests interference with some glucose-requiring organism.

The effect of sulfur-rich protein may be explained by assuming increased biliary excretion of taurine conjugated bile acids. Bile acids occur as taurine or glycine conjugates in the bile, and the free acids are usually recovered from the feces. In the light of present knowledge the role of choline is not amenable to explanation in terms of bile acid formation and excretion, but data concerning this aspect of cholesterol metabolism, namely, effect of choline on bile-acid formation, are sparse.

The effect of fat has been shown to include increased fecal excretion of bile acid (24). Since these data were not obtained by using isotopically labelled cholesterol, the evidence may be circumstantial, but it is convincing. It may be pointed out that fats have a definite effect on microorganisms. In a review of the subject Nieman (42) pointed out the varying effects that saturated and unsaturated fats have on the growth of certain bacteria. The discussion is too complex to reproduce here. Camien and Dunn (43) have demonstrated the inhibitory effect that saturated fatty acids have on the growth of certain lactobacilli and the reversal of this effect by unsaturated fatty acids. Thus it is quite likely that dietary components partly affect cholesterol metabolism by their influence on the growth of intestinal bacteria. Yet another function of intestinal bacteria concerns the conversion of cholesterol to coprostanol (44, 45), a nonreabsorbed metabolic product of cholesterol.

It would appear that one facet of future research on dietary influences on cholesterol metabolism must concern itself with the effect of these dietary factors on the growth and metabolism of the intestinal flora. Such studies interwoven with studies on the effect of intestinal flora on the metabolism of cholesterol, both endogenous and exogenous, should yield some definitive answers concerning the influence of dietary composition on cholesterol metabolism.

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Report of the Uniform Methods Committee

AT A MEETING of the Uniform Methods Committee, in the Sherman hotel, Chicago, on October 20, 1958, the following matters were discussed and the indicated decisions were made. The meeting was attended by six of the seven members of the Uniform Methods Committee. E. M. Sallee, who recently succeeded T. H. Hopper as editor of the methods, was present by invitation as a member *ex officio*. President J. C. Konen was present for part of the meeting.

1. Color Committee—R. C. Stillman, chairman

A.O.C.S. Official Method Cc 13b-45

This method for color of oils and fats by the "Wesson Method Using Lovibond Glasses," has been revised to require calibration of the glasses to conform to the National Bureau of Standards N" scale, or they may be compared against a standard set so calibrated. A complete technical explanation of this decision was published in J.A.O.C.S. for March, 1958 (Vol. *35*, pp. 134–135).

It was suggested that the correct addresses of the Electrical Testing Laboratories and the National Bureau of Standards, by whom the calibration may be performed, be retained in the revised method. Also a source and designation of a paint for the interior of the color booth, which will meet the required Munsell value of 4/, should be included in the method.

With these additions the U.M.C. recommends that the proposed revision be adopted. The method will retain its official status. *Adopted.*

2. Fat Analysis Committee—V. C. Mehlenbacher, chairman

a) *A.O.C.S. Tentative method Ca 2e-55*
Moisture—by Modified Karl Fischer Reagent

At the time of its adoption, several differences between this method, for moisture in oils and fats, and a similar method, Ea 8-56, for moisture in glycerine, were noted; and, in the interest of uniformity of reagents and apparatus, the appropriate committees were requested to explore the possibility of revisions to bring them into more perfect accord. C. L. Hoffpauir, chairman of the moisture subcommittee, has effected the desired revision in a commendable manner. After consultation with the chairman of the Fat Analysis Committee, the U.M.C. decided to include a magnetic or mechanical stirrer in "A-6 Apparatus" and require the use of such a stirrer in the procedures for calibration of iodine-methanol solution and for analysis of samples. Titration in a closed system already is prescribed in the method.

With this change the U.M.C. recommends adoption of this proposed revision. The method will remain "Tentative." *Adopted.*

b) *A.O.C.S. Official Method Cc 12-41, Titer Test*

This method has been revised to require the temperature of the air bath around the sample to be controlled by immersion in a liquid bath maintained at 15° to 20°C. below the expected titer point. An improved dry ice-ethylene glycol bath is employed for samples of titer below 35°C. The change in bath temperature, from the present 20° ± 1°C. for samples of titer 35°C. or higher, has been shown to be without effect on samples of fats of titer from 38°C. to 55°C., and even higher. The proposed revision has been delayed in order to enable these comparisons to be made.

The U.M.C. approves this proposed revision and recommends its adoption. The method will retain its official status. *Adopted.*

c) Subcommittee on Analysis of Commercial Fatty Acids—J. L. Trauth, chairman

1) *Rosin Acids in Fatty Acids, L 14a-58*

A new method, for determination of rosin acids in commercial fatty acids, is proposed. In principle it is similar to Da 12-48, but sulfuric acid is substituted for naphthalene-beta-sulfonic acid in the esterification with methanol.

A few minor additions have been made to amplify specification of apparatus and reagents. In calculation of the percentage of rosin acids, the subtraction of the correction "0.74" is made mandatory instead of implied, as indicated in "Note 1." In the standardization of alcoholic potassium hydroxide, a reference to A.O.C.S. Spec. H 12-52 is added.

With these few changes, which have been accepted by the committee, the U.M.C. approves adoption of this new method as "Tentative." *Adopted.*

2) *Heat Stability Test, L 15a-58*

The proposed method measures the stability of fatty